

**REMARKS**

Applicants have canceled previous claims 1-44 and added new claims 45-57.

Applicants are submitting herewith a request for interference with Patent 6,303,305 B1.

Applicants submit that the newly presented claims are directed to allowable subject matter and respectfully request entry. The claims are supported by the specification as detailed below.

	<b>New Claims</b>	<b>Applicants' Disclosure</b>
45.	A method for quantification of the concentration of a nucleic acid in a sample, comprising the steps of:	Page 19, lines 23-25; Page 65, line 32 through page 66 line 2.
	a) mixing the sample with an amplification reagent;	Page 67, lines 10-25; Page 97, lines 24-26.
	b) amplifying at least one nucleic acid sequence in the sample to create a nucleic acid amplification product;	Page 66, lines 2-29; page 98, lines 14-18.
	c) determining the amount of the nucleic acid amplification product as a function of amplification reaction time;	Page 7, lines 8-14; page 8, lines 10-16; Page 74 line 8 through page 84 line 23.
	d) calculating a derivative of said function;	Page 7, lines 14-15; page 8 line 17; page 10 line 6; page 83 line 32 through page 96 line 22.
	e) determining the maximum of said derivative;	Page 83 line 32 through page 84 line 16; Page 90, lines 11-29; Page 93 line 16 through page 96 line 22.

	f) calculating from said maximum the initial concentration of the nucleic acid sequence in the sample.	Page 96 line 23 through page 99 line 16.
46	The method of claim 45, wherein during one phase of the amplification reaction the amount of amplification product increases progressively and wherein after said progressive phase, the rate of amplification decreases.	Page 83, lines 10-31.
47	The method of claim 45, wherein the amount of the amplification product is determined during a logarithmic growth phase of the amplification.	Page 83, lines 16-31.
48	The method of claim 45, wherein the amplification product is detected by means of fluorescence.	Page 48, lines 5-12.
49	The method of claim 46, wherein the amplification is obtained by a polymerase chain reaction and the amplification product is detected by an intercalating dye.	Page 48, lines 30-32; page 66, lines 2-10.
50	The method of claim 48, wherein amplification is obtained by a polymerase chain reaction and the amplification product is detected by two polynucleotide probes, each labeled with a fluorescent entity, such that when both probes are hybridized to one strand of the nucleic acid amplification product, fluorescence resonance energy transfer occurs between the two fluorescent entities.	Page 49, lines 9-16; page 66, lines 2-10.

51	The method of claim 47, wherein said derivative is calculated by a mathematical fit.	Page 105, lines 20-25.
52	The method of claim 51 wherein the amplification product is detected by means of fluorescence.	Page 48, lines 5-12.
53	The method of claim 51, wherein the amplification is obtained by a polymerase chain reaction and the amplification product is detected by an intercalating dye.	Page 48, lines 30-33; page 66, lines 2-10.
54	The method of claim 45, wherein the step of calculating the derivative of said function comprises calculating the second derivative.	Page 83 line 32 through page 84 line 16.
55	The method of claim 45, wherein the step of calculating the derivative of said function comprises calculating, the first derivative.	Page 93 line 16 through page 96 line 22.
56.	A method for quantification of the concentration of a nucleic acid in a sample, comprising the steps of:	Page 19, lines 23-25; Page 65, line 32 through page 66 line 2.
	a) mixing the sample with an amplification reagent;	Page 67, lines 10-25; Page 97, lines 24-26.
	b) amplifying at least one nucleic acid sequence in the sample to create a nucleic acid amplification product;	Page 66, lines 2-29; page 98, lines 14-18.

	c) determining the amount of the nucleic acid amplification product as a function of amplification reaction time;	Page 7, lines 8-14; page 8, lines 10-16; Page 74 line 8 through page 84 line 23.
	d) calculating the second derivative of said function;	Page 83 line 32 through page 84 line 16.
	e) determining the maximum of said second derivative; and	Page 83 line 32 through page 84 line 16; Page 90, lines 11-29
	f) calculating from said maximum the initial concentration of the nucleic acid sequence in the sample.	Page 10 lines 9-17; Page 96 line 23 through page 99 line 16.
57	A method for quantification of the concentration of a nucleic acid in a test sample, comprising the steps of:	Page 19, lines 23-25; Page 65, line 32 through page 66 line 2.
	a) mixing the test sample with an amplification reagent;	Page 67, lines 10-25; Page 97, lines 24-26.
	b) amplifying at least one nucleic acid sequence in the test sample by a process comprising the step of subjecting the test sample to a number of amplification cycles to create a nucleic acid amplification product;	Page 66, lines 2-13;
	c) determining a value corresponding to the relative amount of the nucleic acid amplification product for each amplification cycle to generate a data set;	Page 7, lines 8-18; Page 74 line 8 through page 84 line 23;
	d) generating a function from said data set;	Page 83 line 10 through page 84 line 23; Page 105, lines 20-25.

	e) calculating a derivative of said function;	Page 7, lines 14-15; page 8 line 17; page 10 line 6; page 83 line 32 through page 84 line 23; Page 105, lines 20-25.
	f) determining a fractional cycle number corresponding to a maximum of said derivative;	Page 7 lines 16-32; page 83 line 32 through page 84 line 23;
	g) obtaining a calibration curve generated using steps a-e on a plurality of additional nucleic acid calibration samples, each additional calibration sample having a known concentration of the nucleic acid sequence; and	Page 97 line 13 through page 99 line 16.
	h) determining the initial concentration of the nucleic acid sequence in the test sample using the calibration curve.	Page 96 line 23 through page 99 line 16.

Entry of the amendment is requested.

Respectfully submitted,

By: Mark B. Floyd  
Mark B. Floyd  
Agent for Applicants  
Registration No. 41,022

Date: October 16, 2002

Cepheid  
904 Caribbean Drive  
Sunnyvale, CA 94089  
**Direct Dial: (408) 400-8237**  
Facsimile: (408) 734-1260

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE TITLE:**

Please change the TITLE to:

METHOD [COMPUTER PROGRAM PRODUCT] FOR QUANTITATIVE ANALYSIS OF A  
NUCLEIC ACID AMPLIFICATION REACTION

**IN THE SPECIFICATION:**

Please replace the paragraph beginning on page 1 lines 24-26 with the following paragraph:

--This invention relates to a method and computer program product for quantitative analysis of a nucleic acid amplification reaction.--

**IN THE CLAIMS**

**Please cancel claims 1-44 and Add new claims 45-57.**

45. (new) A method for quantification of the concentration of a nucleic acid in a sample, comprising the steps of:
- a) mixing the sample with an amplification reagent;
  - b) amplifying at least one nucleic acid sequence in the sample to create a nucleic acid amplification product;
  - c) determining the amount of the nucleic acid amplification product as a function of amplification reaction time;
  - d) calculating a derivative of said function;
  - e) determining the maximum of said derivative; and
  - f) calculating from said maximum the initial concentration of the nucleic acid sequence in the sample.

46. (new) The method of claim 45, wherein during one phase of the amplification reaction the amount of amplification product increases progressively and wherein after said progressive phase, the rate of amplification decreases.
47. (new) The method of claim 45, wherein the amount of the amplification product is determined during a logarithmic growth phase of the amplification reaction.
48. (new) The method of claim 45, wherein the amplification product is detected by means of fluorescence.
49. (new) The method of claim 46, wherein the amplification is obtained by a polymerase chain reaction and the amplification product is detected by an intercalating dye.
50. (new) The method of claim 48, wherein amplification is obtained by a polymerase chain reaction and the amplification product is detected by two polynucleotide probes, each labeled with a fluorescent entity, such that when both probes are hybridized to one strand of the nucleic acid amplification product, fluorescence resonance energy transfer occurs between the two fluorescent entities.
51. (new) The method of claim 47, wherein said derivative is calculated by a mathematical fit.
52. (new) The method of claim 51 wherein the amplification product is detected by means of fluorescence.
53. (new) The method of claim 51, wherein the amplification is obtained by a polymerase chain reaction and the amplification product is detected by an intercalating dye.

54. (new) The method of claim 45, wherein the step of calculating the derivative of said function comprises calculating the second derivative.
55. (new) The method of claim 45, wherein the step of calculating the derivative of said function comprises calculating, the first derivative.
56. (new) A method for quantification of the concentration of a nucleic acid in a sample, comprising the steps of:
- a) mixing the sample with an amplification reagent;
  - b) amplifying at least one nucleic acid sequence in the sample to create a nucleic acid amplification product;
  - c) determining the amount of the nucleic acid amplification product as a function of amplification reaction time;
  - d) calculating the second derivative of said function;
  - e) determining the maximum of said second derivative; and
  - f) calculating from said maximum the initial concentration of the nucleic acid sequence in the sample.
57. (new) A method for quantification of the concentration of a nucleic acid in a test sample, comprising the steps of:
- a) mixing the test sample with an amplification reagent;
  - b) amplifying at least one nucleic acid sequence in the test sample by a process comprising the step of subjecting the test sample to a number of amplification cycles to create a nucleic acid amplification product;
  - c) determining a value corresponding to the relative amount of the nucleic acid amplification product for each amplification cycle to generate a data set;
  - d) generating a function from said data set;
  - e) calculating a derivative of said function;



- f) determining a fractional cycle number corresponding to a maximum of said derivative;
- g) obtaining a calibration curve generated using steps a-e on a plurality of additional nucleic acid calibration samples, each additional calibration sample having a known concentration of the nucleic acid sequence; and
- h) determining the initial concentration of the nucleic acid sequence in the test sample using the calibration curve.